STUDIES ON THE THERMAL DEATH OF HYALELLA AZTECA SAUSSURE [1]

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Introduction

Much work has been done in the past half century concerning the effects of temperature on the survival of organisms. However, little has been done in that respect with the Crustacea, particularly the fresh water forms.

A study was made from June 16, 1947, to August 15, 1947, concerning the effects of temperature on the survival and death rates of the amphipod *Hyalella azteca* Saussure (*Hyalella knickerbockeri* Bate, *Hyalella dentata* Smith). This amphipod is abundant in the waters of Little Miller's Bay in West Okoboji Lake, Dickinson County, Iowa, at the site of the Iowa Lakeside Laboratory, where the investigation was conducted.

Historical Survey

Geisler (1944) suggests that rate of development in *Hyalella azteca* is directly related to the temperature, but does not record the effects of higher temperatures. For marine copepods and decapods, Huntsman and Sparks (1924) report heat death at temperatures between 22° and 33° C. when the animals were exposed to temperatures rising at the average rate of 0.2° C. per minute. Brown (1928) reported a temperature characteristic, μ, of 187,000 calories from 35° to 41° C. for thermal death in *Daphnia magna*.

Materials and Methods

Numerous Hyalella were secured daily by placing fresh masses of the green alga, *Cladophora fracta,* in which they feed, in a pail of lake water. Amphipods which came to the surface of the water were transferred to a stock tank of fresh lake water at 20° to 22° C. by means of a tea strainer. This lake water was partially changed daily and replaced weekly, and was used in all experiments. The temperature of the tank water was about the same as that of the natural water where the animals were taken.

The water baths used in the experiments were five-gallon containers heated electrically and controlled manually to $\pm 0.2°$ C.

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In short exposures several hundred Hyalella were dipped from the stock tank with a tea strainer, over which a muslin square was then fastened and held taut with a rubber band. The strainer was immersed and oscillated in the water bath for the desired period. Then the Hyalella and the strainer were transferred quickly to enamelled basins of fresh lake water at 20° to 22° C.

In exposures longer than thirty seconds small tin cans with tops and bottoms removed were employed. Muslin squares fastened over the open ends kept the amphipods confined, but exposed to the water. A number of these tins were placed in the water bath and removed as desired.

The water bath was stirred between short exposures, and during exposures longer than thirty seconds was continually aerated with compressed air. The tins containing the Hyalella were placed on a wire platform as near the center of the water bath as possible, and at least six inches away from the source of heat.

Exposed animals were allowed to remain in recovery basins four or more hours before counting. Counting was usually done six to eight hours after exposure. Check counts showed that injured animals which were alive four hours after exposure were always dead after sixteen hours. Therefore, injured animals were counted as dead. Control animals remained alive, except for rare exceptions, after twenty-four hours in the basins.

Water from each basin was individually strained through muslin to collect the the amphipods for counting. The muslin was spread flat on a moist, concrete slab for counting under a sixty-watt electric bulb. Because of negative tropism to light (Phipps, 1915), the living amphipods crawled toward the periphery. A few cubic centimeters of water dropped at the center of the muslin hastened the outward movement. After thus separating the living and dead, the living were counted first. Those evidencing injury or feebleness when stimulated with water or the points of a pair of tweezers were counted as dead.

Mature specimens were separated into two groups; so also were immature specimens. Those longer than 7 mm. were considered very large adults; those from 3½ to 7 mm. as large adults; those from 2½ to 3 mm. as medium-sized adolescents; and those still less as small and juvenile (Geisler, 1944). A separate count was taken according to sexes for each of the adult groups on the basis of salient characteristics (Geisler, 1944) as seen through a dissecting scope.

Time intervals were determined with a watch calibrated in fifths of a second.

Experimental Results

Survival in constant temperature baths

Constant temperatures used included one degree intervals C. from 38° to 50°. Tests were also run at 36.5°, 35° and 33°.

Thermal death times and temperatures here shown are those at which fifty percent of the organisms survived.

At 50°, less than one second was sufficient time to cause thermal death. At lower temperatures the time increased gradually so that at 40° an exposure of 75 seconds was needed. Below 40° the increase in time required to kill was very sharp, so that at 33° more than eleven hours (39,600 seconds) was necessary (Table 1).

Size and survival in constant temperature baths

Age and size are directly correlated in Hyalella; the larger the animal, the older it is (Geisler, 1944). Large adults showed the greatest resistance, usually higher than the average figure for the total of all simultaneously exposed. Very large adults varied in their resistance, but their rate of survival usually approached the average. Medium adolescents showed still less resistance; and small juveniles were least resistant (Table 1).

TABLE 1

Heat death for Hyalella azteca in constant temperature baths

Degrees C.	Time exposed in seconds	Per cent survivors					Total number animals exposed
		Total animals exposed	Very large adults	Large adults	Medium adolescents	Small juveniles	
50.0	1	50.8	52.1	62.5	45.6	43.3	240
49.0	4	51.2	64.8	59.4	47.5	44.4	416
48.0	7	50.3	48.7	51.8	52.0	47.6	320
47.0	8	50.8	58.3	57.2	48.2	46.6	114
46.0	12	49.3	31.7	51.9	54.3	47 1	162
45.0	14	47.1	52.2	65.4	36.3	43.6	318
44.0	15	55.0	40.1	56.2	56.7	49.4	623
43.0	24	49.5	58.5	63.6	43.0	43.3	656
42.0	39	49.9	52.8	56.4	49.1	37.9	204
41.0	60	47.6	33.6	67.6	51.3	46.1	432
40.0	75	49.6	49.9	68.8	47.6	46.8	626
39.0	135	49.3	49.3	58.4	44.6	48.4	636
38.0	240	48.9	49.2	55.8	45.0	46.9	547
36.5	1,800	49.9	44.8	52.9	51.1	53.6	210
35.0	6,300	51.8	50.2	56.2	46.1	46.8	583
33.0	39,600	53.7	51.2	51.9	51.5	48.2	146

Sex and survival in constant temperature baths

No valid evidence was found to indicate that sex affects resistance to heat in constant temperature baths. For all adult specimens, male survivors outnumbered females in sixteen out of thirty-two cases.

Survival in rising temperature baths

Using the same equipment as that employed for the constant temperature baths, Hyalella were exposed to four average rates of temperature rise, beginning at 20° to 22° C. Rates of rise were: 0.375° per minute; 0.261° per minute; 0.150° per minute; and 0.036° per minute. At lower temperatures the rate of rise per degree C. was more rapid than at higher temperatures. The rates of rise are here expressed as average rates, in order to make them comparable to those of other investigators who previously encountered the same difficulty (Huntsman and Sparks, 1924). Within the rates of rise investigated, thermal death did not occur below 39° C. and always was found above 41° C. (Table 2).

TABLE 2

Heat death in Hyalella azteca in rising temperature baths

Rate* of rise in degrees C. per minute from 20° to 22° as a base	Degrees C.	Total animals exposed	Per cent survivors				
			Total animals	Very large adults	Large adults	Medium adolescents	Small juveniles
0.375	37.0	178	89.4	94.6	92.6	88.8	74.1
	38.0	212	84.4	85.7	77.8	75.8	75.0
	39.0	189	87.8	93.7	88.8	82.7	78.3
	40.0	201	51.7	52.7	50.2	50.0	51.8
	41.0	170	60.2	69.7	54.8	52.6	50.0
	42.0 to 45.0	#200@	....	....	....	....	....
0.261	37.0	462	85.9	95.5	96.3	89.1	83.1
	38.0	470	81.1	91.1	83.7	77.2	76.7
	39.0	456	83.9	93.1	83.1	83.7	78.6
	40.0	534	68.1	81.0	79.9	63.1	64.6
	41.0	340	27.6	31.2	23.6	26.5	29.0
	42.0 to 45.0	#400@	....	....	....	....	....
0.150	37.0	421	93.5	96.4	95.8	86.5	87.5
	38.0	492	82.9	92.3	85.8	76.8	77.1
	39.0	493	77.4	88.6	76.9	75.4	66.6
	40.0	430	70.7	75.0	73.0	65.3	65.5
	41.0 to 45.0	#400@	....	....	...	....	....
0.036	36.0	464	94.8	97.6	96.0	89.6	91.8
	37.0	306	86.9	94.6	95.6	82.5	78.7
	38.0	493	65.9	73.5	81.5	59.8	55.9
	39.0	194	29.3	12.7	39.0	42.0	25.6
	40.0 to 45.0	#350@	....	....	....	....	....

* Average rate of rise.

\# Approximate number at each degree of temperature within the bracketed limits; no accurate count taken since all were dead.

Size and resistance to rising temperatures

Very large adults demonstrated the highest resistance in rising temperature baths, except at the slowest rate of rise. Large adults, medium adolescents, and small juveniles showed, respectively, less resistance (Table 2).

Sex and resistance to rising temperatures

No evidence was found that sex causes any variance in resistance to rising temperatures. Male adult survivors in some trials outnumbered females, and vice versa, but never in significant numbers.

Adjustment to rising temperatures of water

A temporary adjustment to rising temperatures was noted. For example, organisms plunged into and continuously exposed to a pre-heated constant temperature bath at 38° C. reached thermal death in four minutes. Within the rates of rise investigated, thermal death was not found to occur at 38°, 87.8 per cent still surviving at that temperature at the fastest rate of rise, and 65.9 per cent surviving at the slowest rate of rise (Table 2).

Temperature coefficients for thermal death

Adaptations of the v'ant Hoff-Arrhenius equation are often used to express the rate of progress in biological reactions, although it is possible that such characteristics are more descriptive than analytically accurate.

Computation and comparison of Q_{10} for a number of temperature ranges within the full range investigated revealed that although death occurs more quickly at higher temperatures, the rate at which the lethal effect progresses decreases as the temperature increases. The decrease in Q_{10} was very marked for intervals below 40° C. Above that temperature there was a sharp break in the deceleration of the rate of progress and the Q_{10} variance was not so great (Table 3).

TABLE 3

Temperature characteristics for Hyalella azteca for thermal death in constant temperature baths

Temperature intervals in degrees C.	Q_{10}
33.0–35.0	9,768.00
35.0–40.0	7,056.00
38.0–43.0	100.00
40.0–45.0	28.91
43.0–48.0	11.75
45.0–50.0	196.00*

* Apparent divergence may be due to experimental inaccuracies.

SUMMARY

1. Thermal death occurs in *Hyalella azteca* at constant temperatures from 33° to 50° C. The time required to produce thermal death varies from more than eleven hours (39,600 seconds) at 33°, to less than one second at 50° C.

2. Comparison of Q_{10} values for narrow ranges within the broad range of temperatures investigated indicates a marked decrease of Q_{10} values at higher temperatures in spite of a more rapid lethal effect.

3. Thermal death occurred in rising temperatures, the slower the rate of rise, the lower the killing temperature, being not below 39° nor above 41° for the rates of rise investigated.

4. A temporary adjustment was found to occur to rising temperatures, delaying thermal death at a given temperature for some time past the period necessary to kill on immersion in the constant temperature bath at the given temperature.

5. Resistance to the effects of heat appears to be directly related to the size and age of the animal, the older and larger the animal the greater the resistance, except for the largest animals (which might have reached a state of senility).

6. Resistance to the effects of heat does not appear to be related to sex in *Hyalella azteca*.

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